

Designer Affinity Reagents

Brian Kay

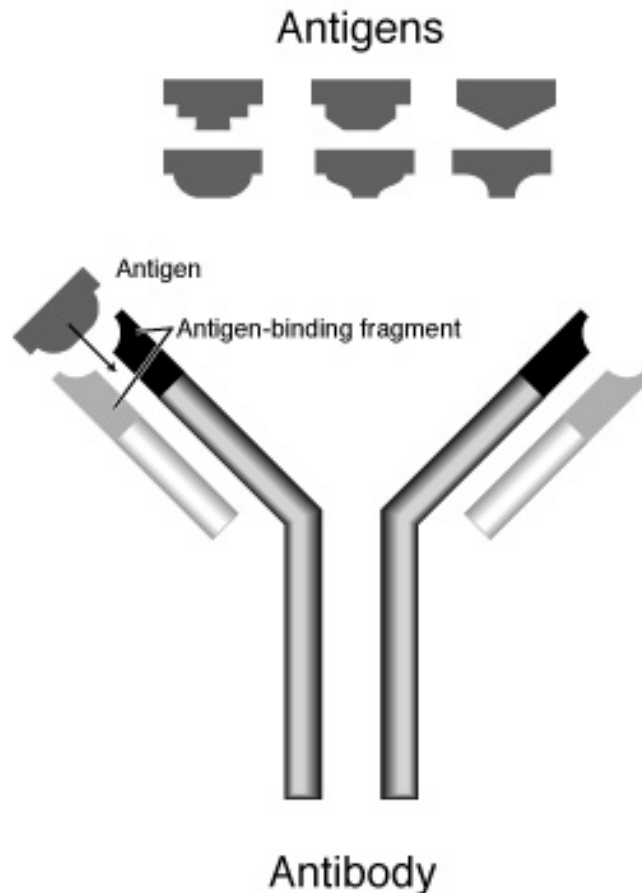
3/6/02

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Applications of Designer Antibodies

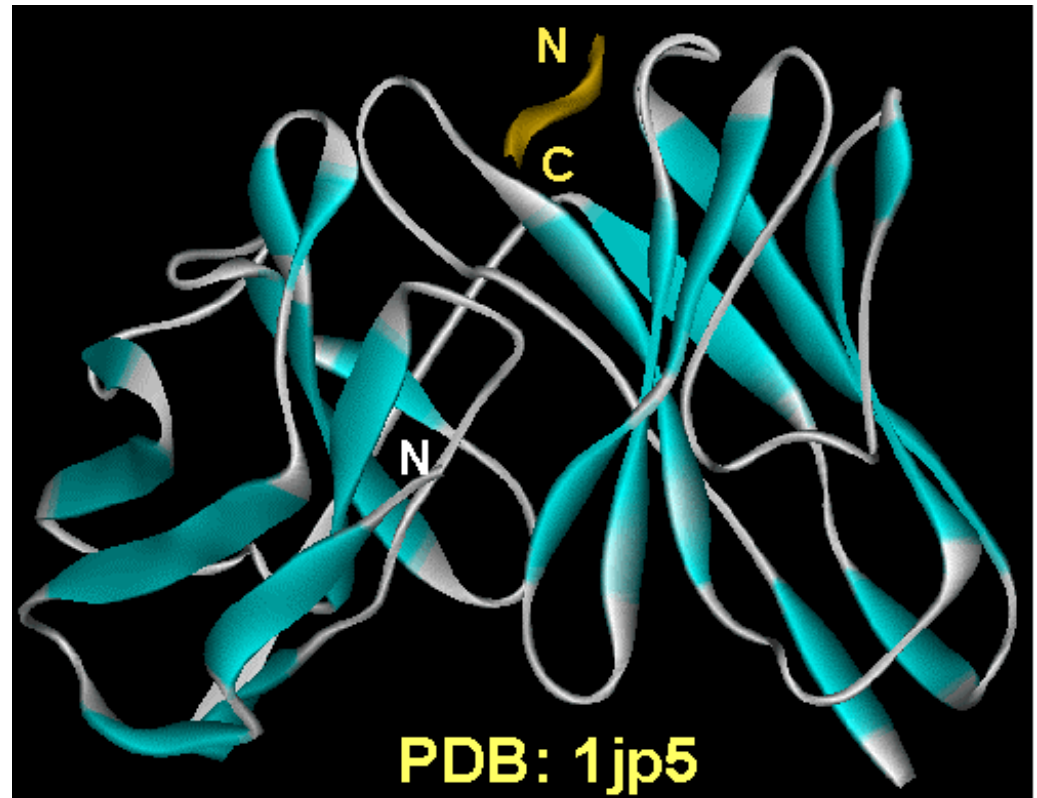
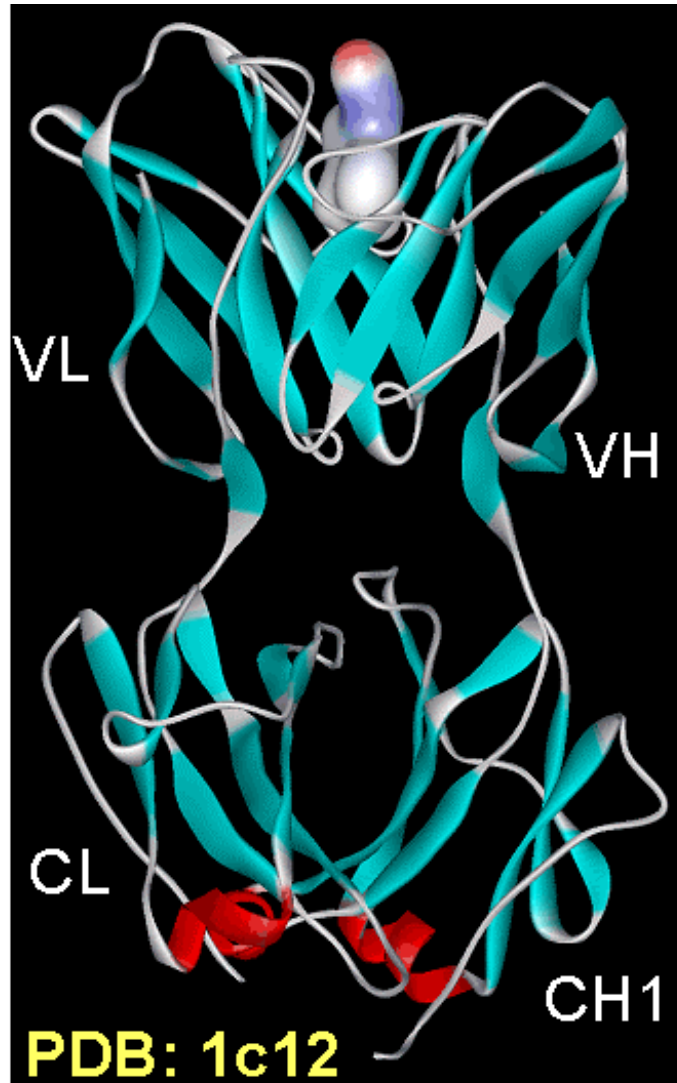
- Designer affinity reagents
 - **Generate antibodies quickly to targets of interest**
 - **Measure the size and amount of target proteins**
 - **Purify the target and associated proteins**
 - **Target cancer cells for detection or therapy**
 - **Organize molecules**
- Promote crystallization of proteins
- Functional genomics

General Structure of Antibodies

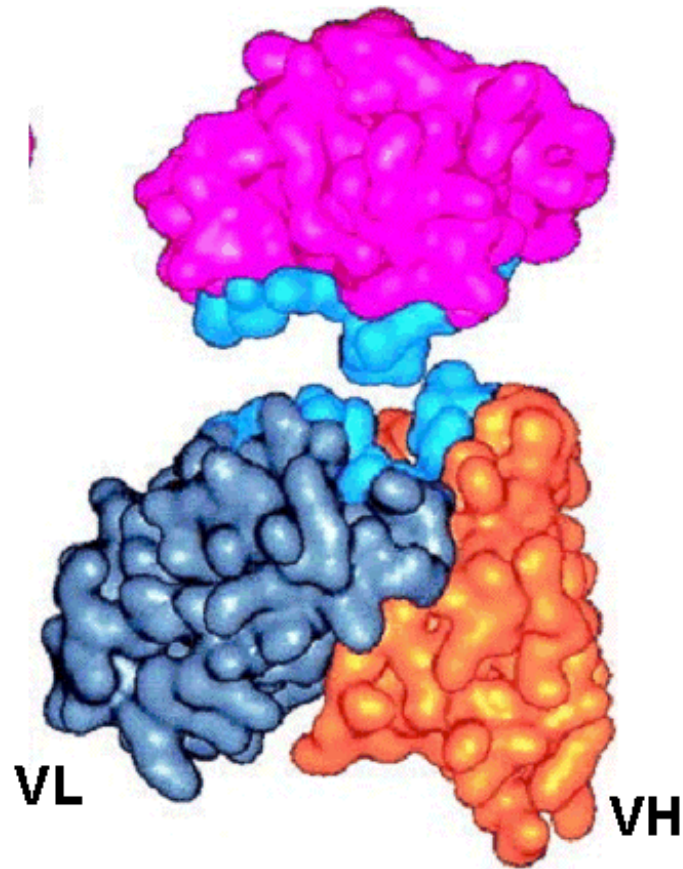


- Affinity reagents with dissociation constants of 10 nM to 1 nM
- Can be generated through immunization of animals, hybridoma production, phage-display, or ribosome display

Fab-odorant scFv-HIV protease peptide

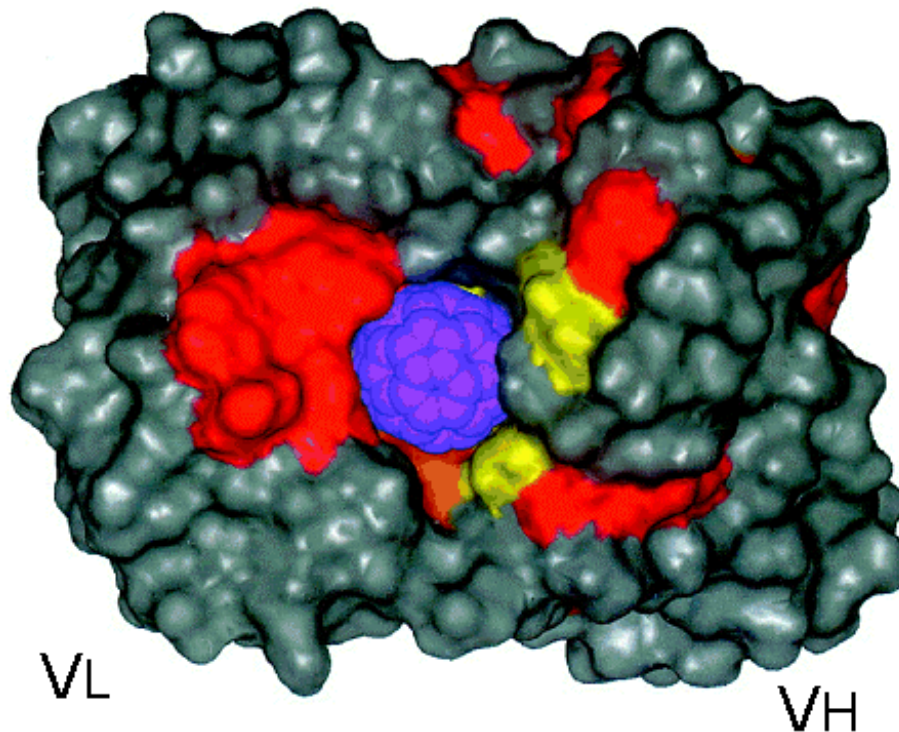


scFv Complexed with Lysozyme

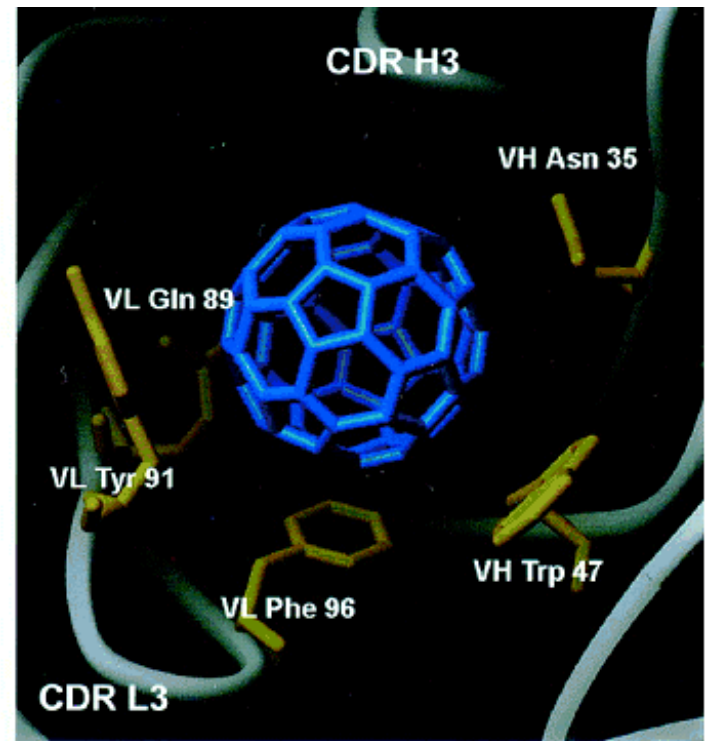


Human scFv

Model of Antibody Complexed with C60

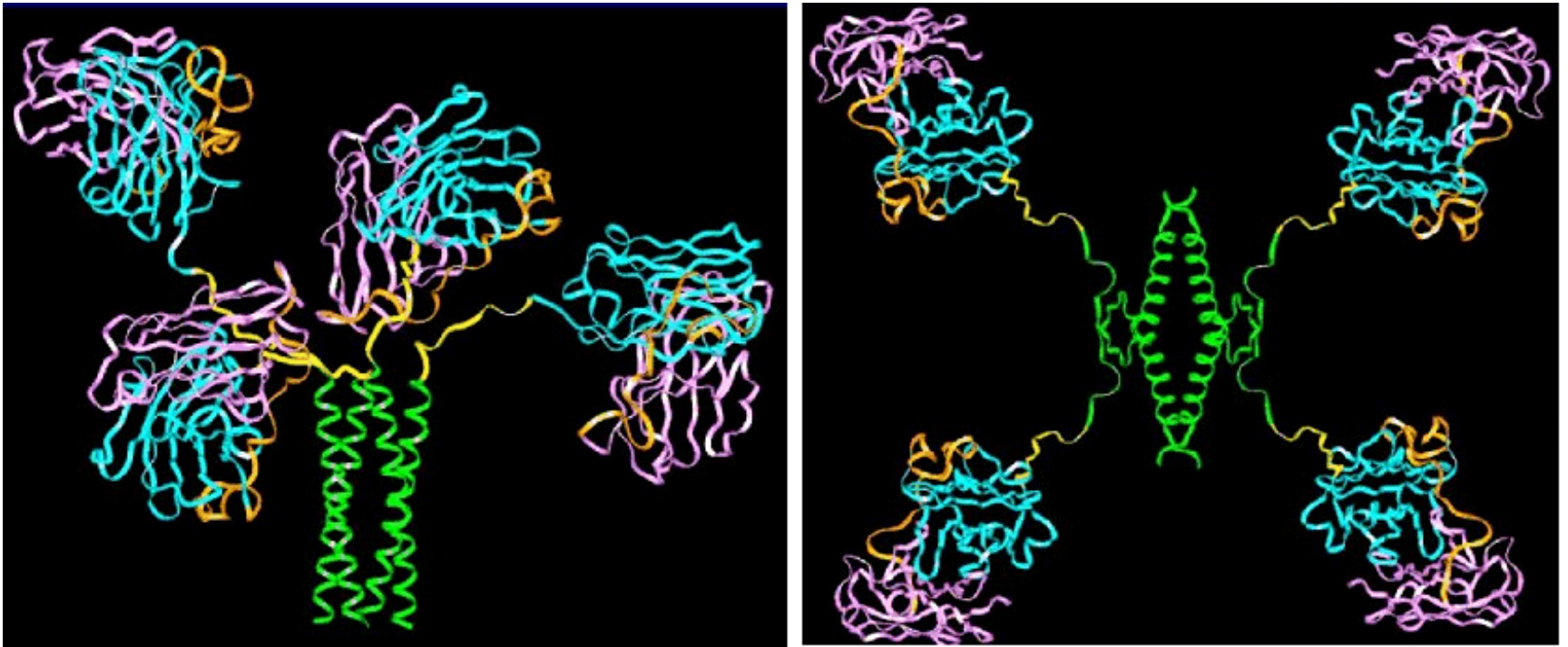


Red = tyrosine
Orange = phenylalanine
Yellow = tryptophan



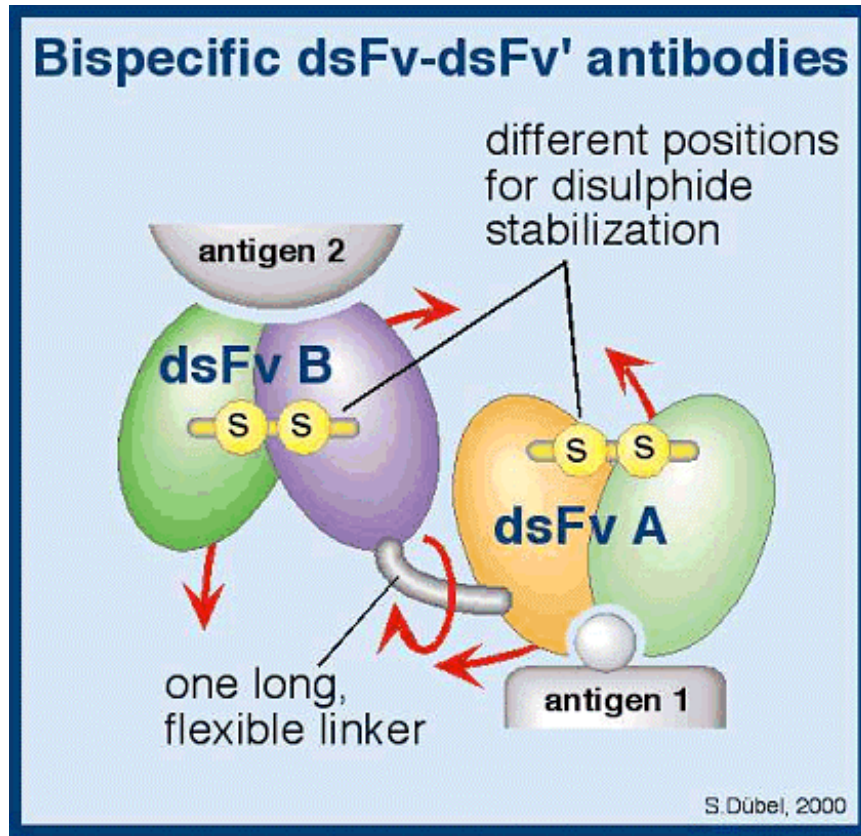
PNAS (2000) 97, 12193-7

Tetrameric Antibodies



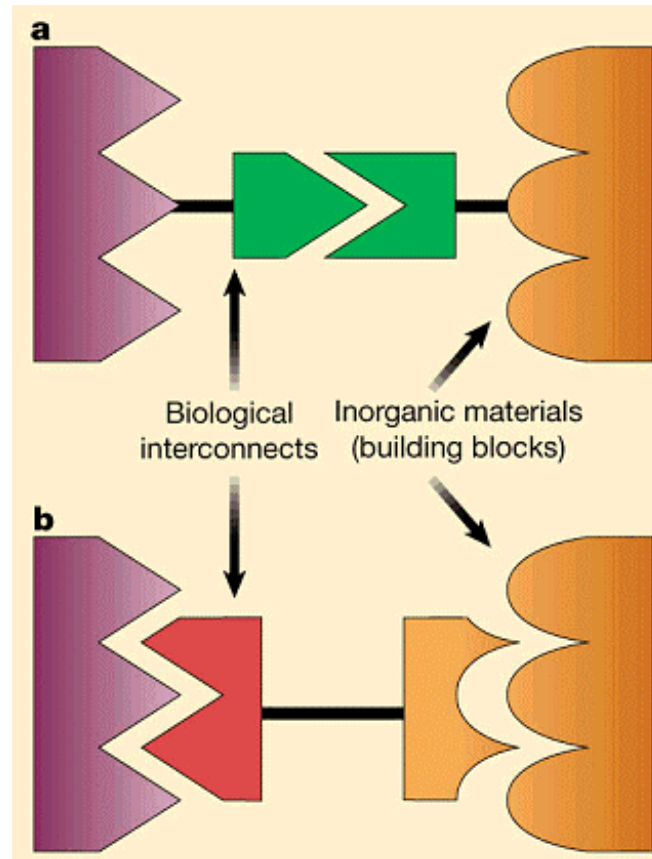
Andreas Plückthun (Zürich)

Diabodies: Bispecific Antibodies



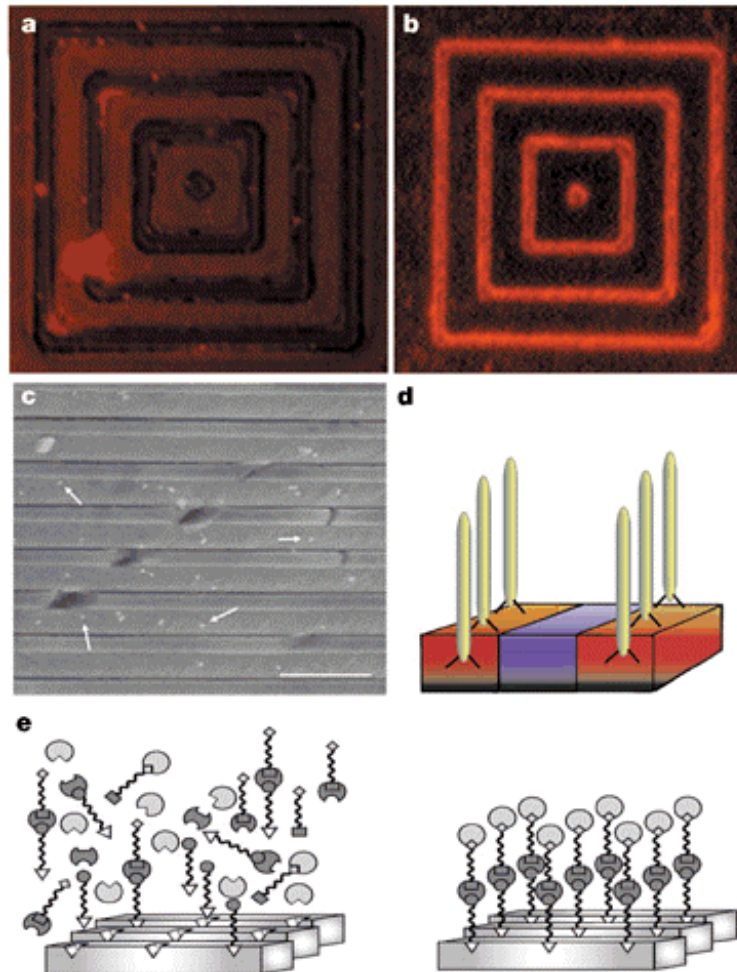
- Diabodies directed to a target cell-surface antigen and a T-cell have shown to be effective in recruiting T-cells to kill cells bearing the target antigen.
- <http://www.mgen.uni-heidelberg.de/SD/S-Duebel.html>

Biological Adaptors



Nature (2000) 405: 626-627.

Building Nanostructures



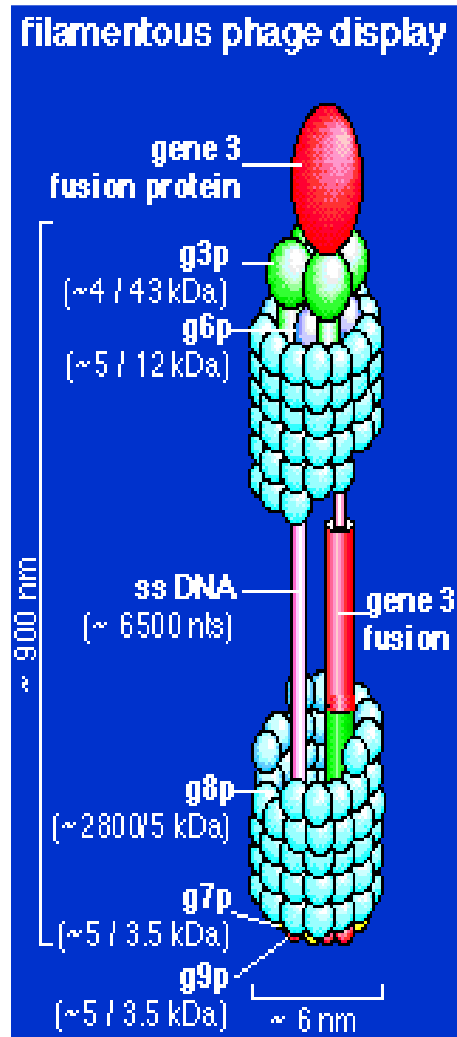
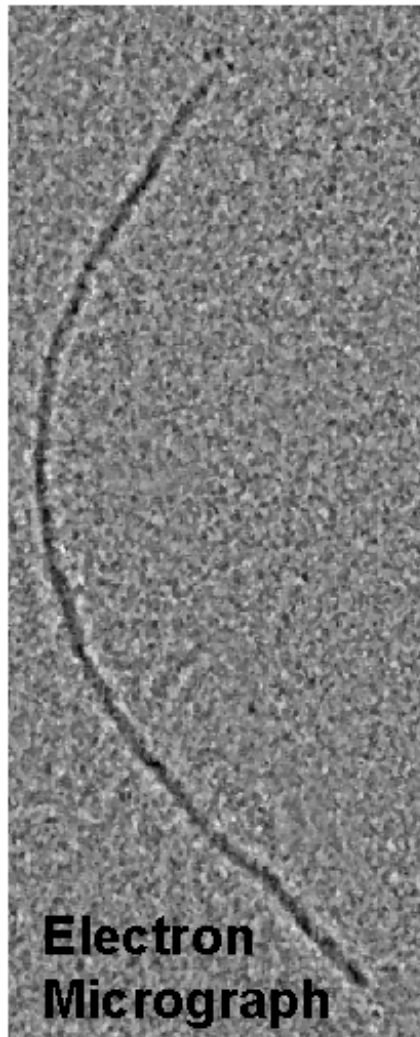
Binding
to GaAs
lines
and not
SiO₂
spaces.

Nature (200)
405: 665-668.

Naïve scFv Library

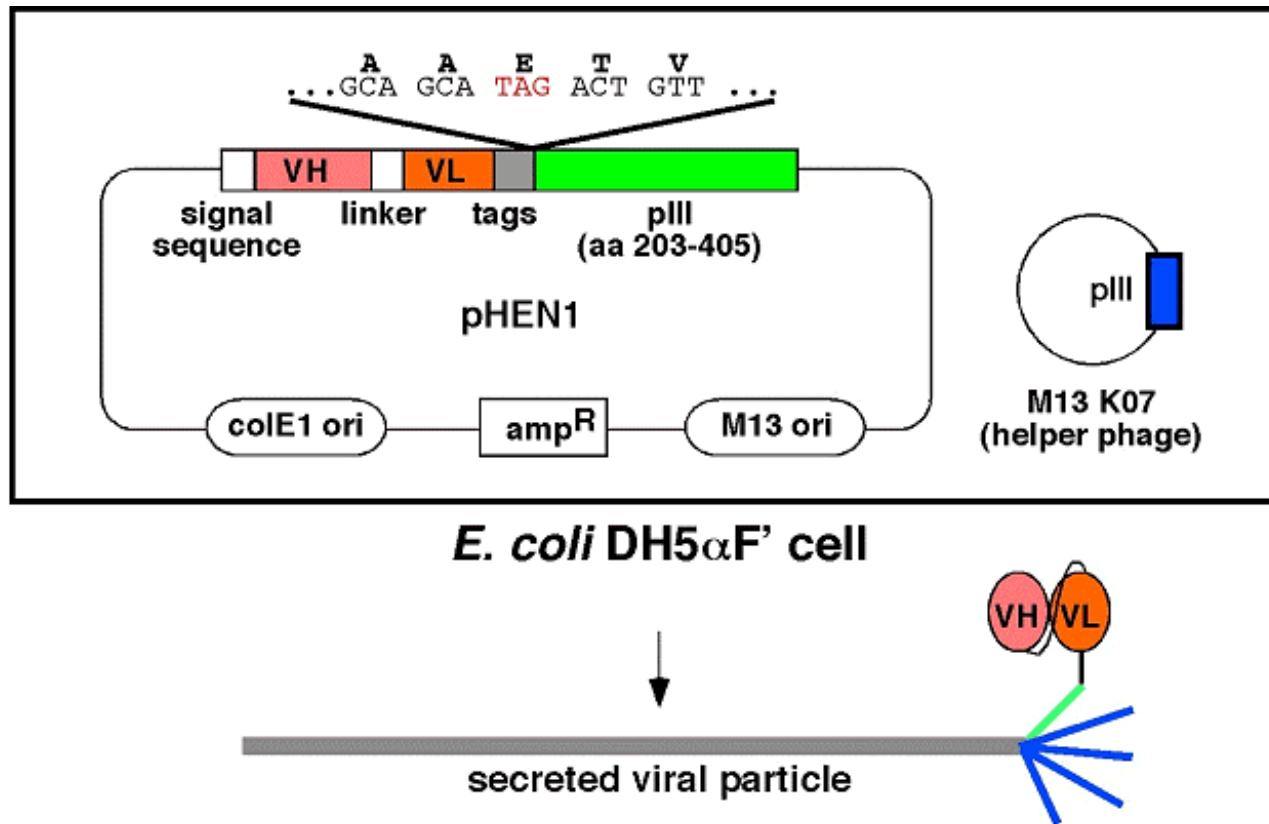
- Displayed on surface of bacteriophage M13
- Constructed by Mike Sheets & Jim Marks
- Human antibody chains
- 6×10^9 recombinants
- Can select 4-10 antibodies to most targets *in vitro*
 - Host protein, cancer markers, etc.
- Typical K_d is 10 nM

Advantages of Phage-Display



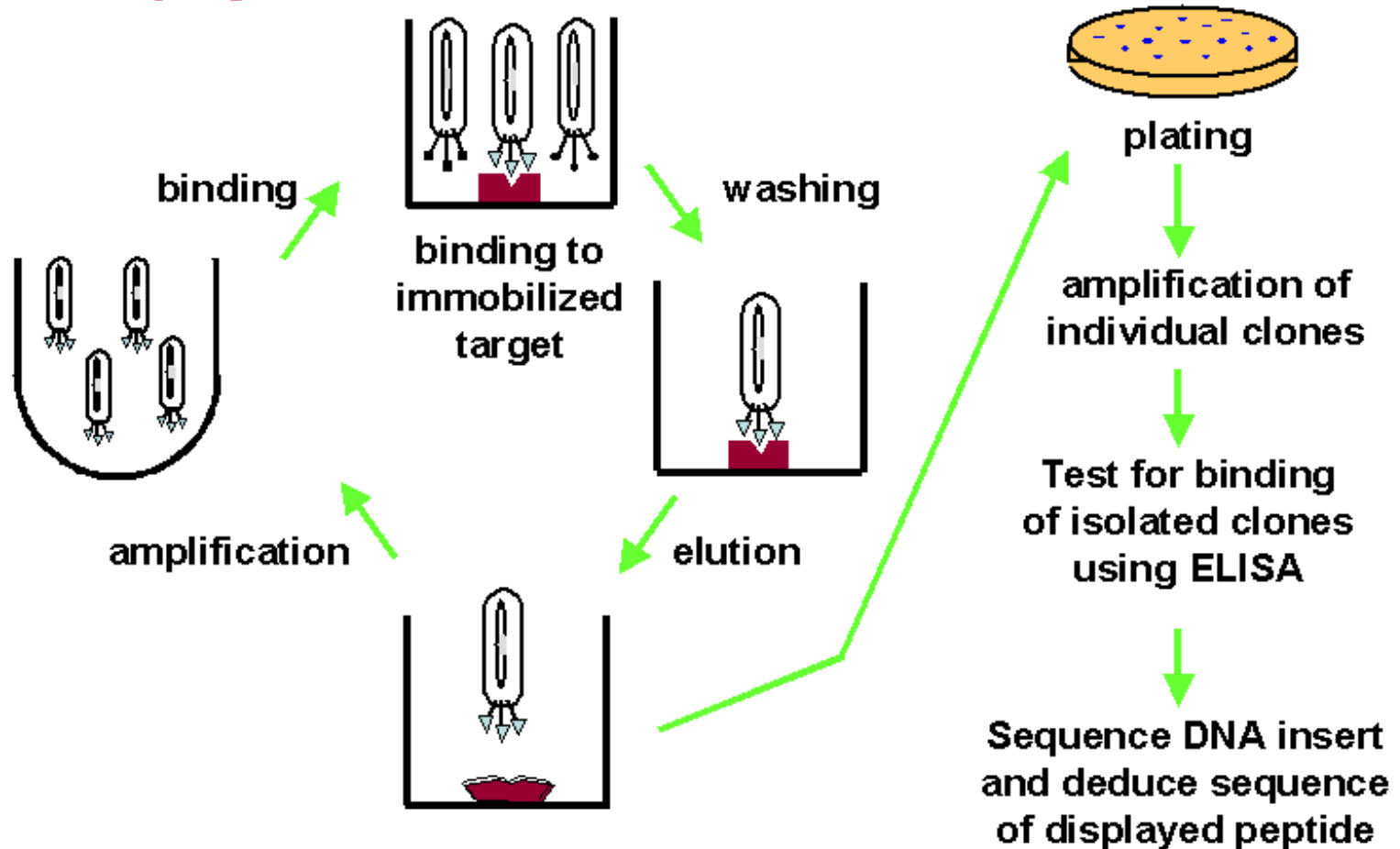
- Can screen large numbers (i.e., 10^{10}) of clones
- Can control the conditions of selection
- Capsid proteins assemble in the periplasmic space which allows for disulfide bond formation

Generation of Phage Particles

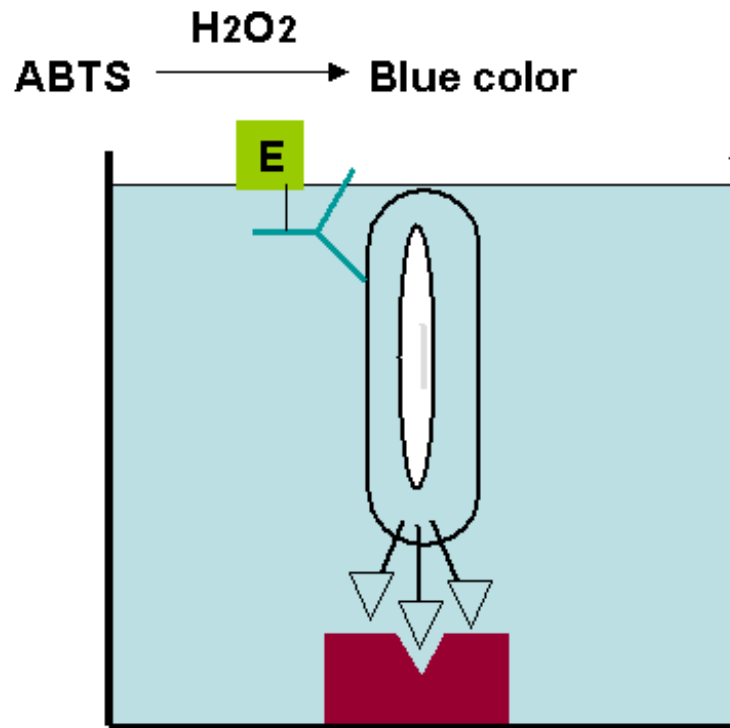


Phage-Display Selection Experiments

**Viral particles expressing
antibody fragments**

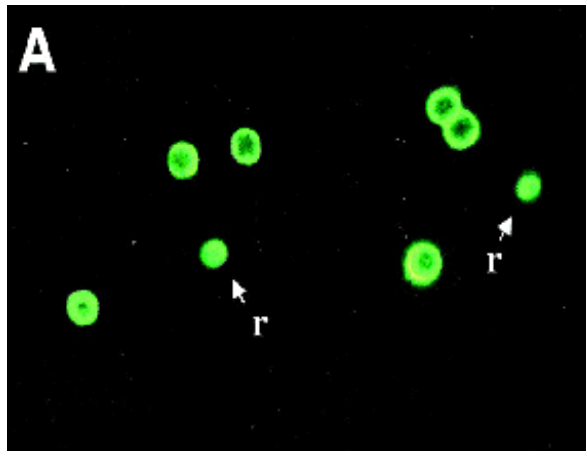


Phage ELISA

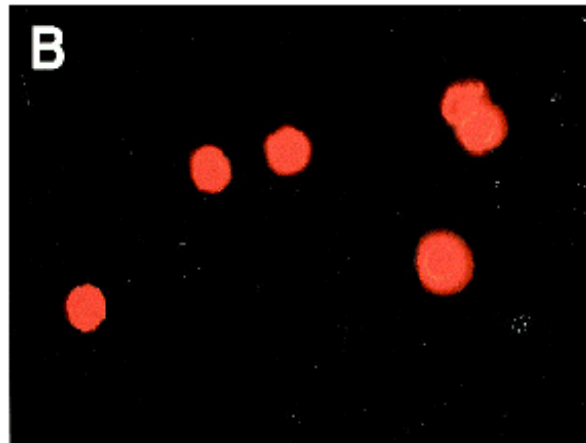


- Method of confirming that isolated phage bind to their target
- Utilizes a monoclonal antibody to the major capsid protein
- Colorimetric readout indicative of the strength of binding

Antibodies to Human Fetal Erythroid Cells



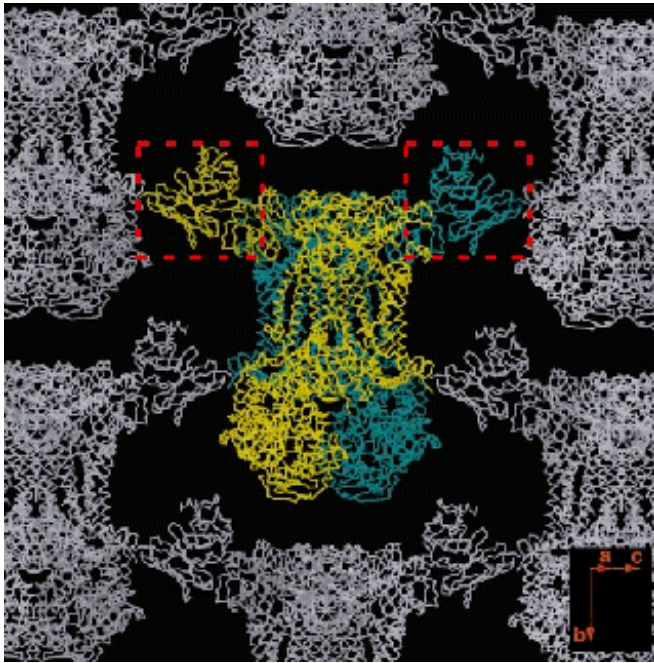
Anti-hemoglobin



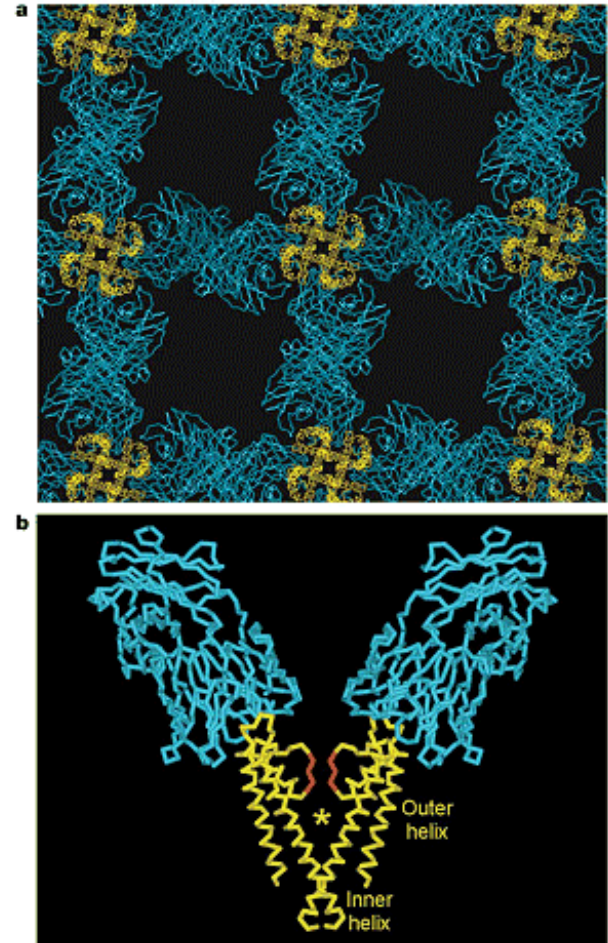
Selected antibody

- Deplete library of antibodies that bind to adult RBC's and WBC's
- Affinity select with fetal, nucleated erythroid cells from fetal liver
- Identify binding phage by FACS
- Identify subset that bind nucleated RBC's
- PNAS (2001) 98, 2682

Membrane Protein-Antibody Crystals



Cytochrome bc1 -Fv complex

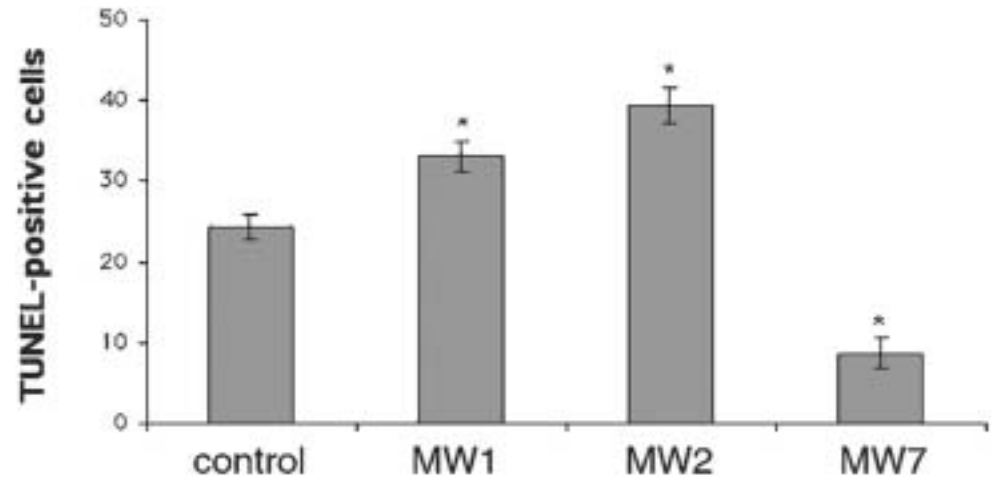
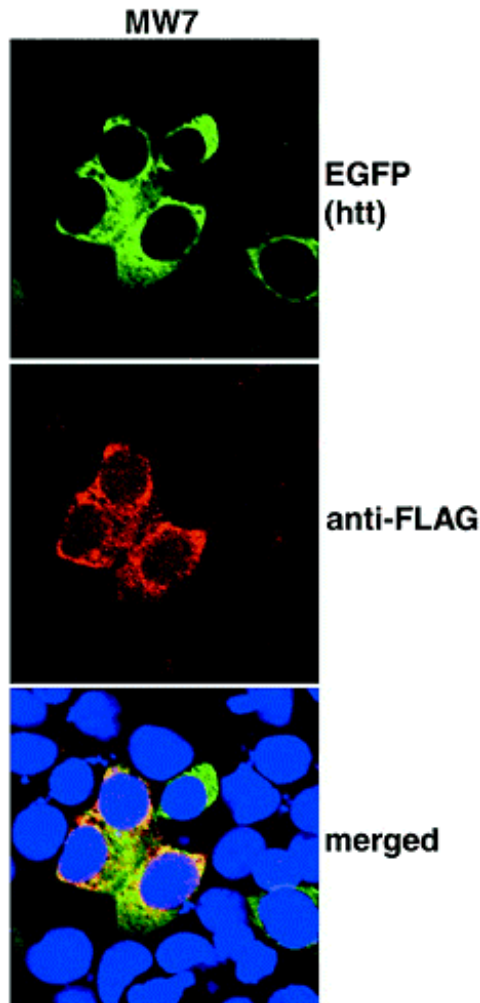


K⁺-channel-Fab complex

Intracellular Antibodies

- Select by phage-display
- Select subset of antibodies that will work in the cytoplasm by yeast two-hybrid screening
- Induce expression in cells and examine the phenotype
- Misdirect target protein with trafficking tags on the antibodies (Nuclear localization signal, nuclear export signal, ER retention signal, myristoylation signal, etc.)
- Ablate bound antigens with laser

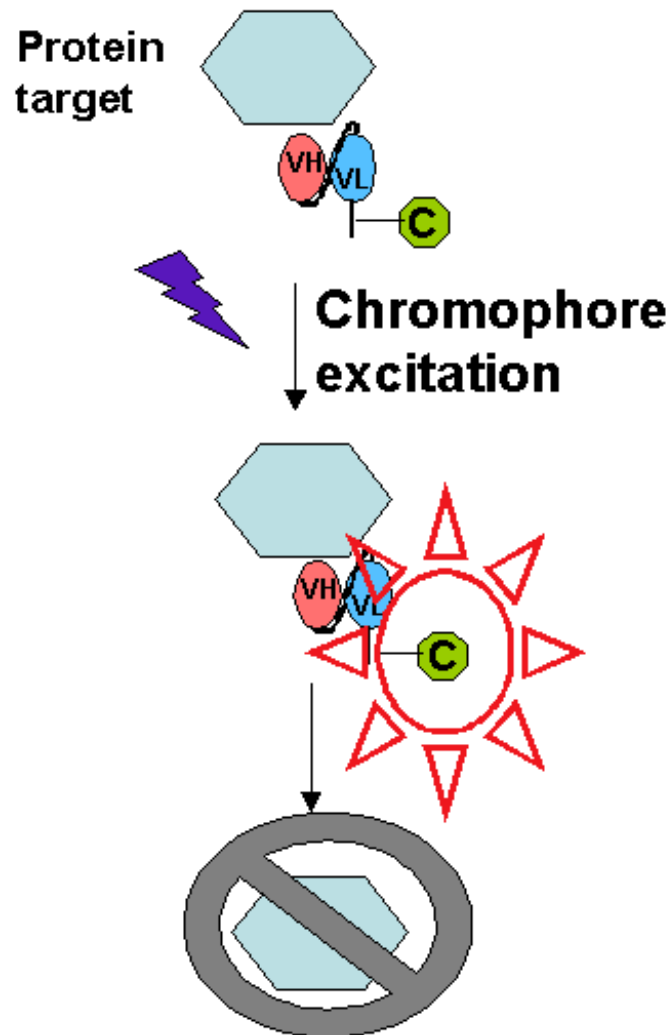
Intracellular Antibody to Huntington Protein



MW7 inhibits aggregation as well as cell death induced by mutant huntington protein (40 Q's).

PNAS (2002) 99, 1002-1007

XCALibur™



- Chromophore assisted laser inactivation (CALI)
- Xerion Pharmaceuticals
- Select antibody via phage-display, label, inject into cells, & shine laser
- Denaturation of associated antigen
- Examine cellular phenotype